PTO 1542
(4 85)

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

ONLINE SEARCH REQUEST FORM

USER R. Weisser SERIAL NUMBER 230108

ART UNIT 182 PHONE 3434 DATE 3/16/89

Please give a detailed statement of requirements. Describe as specifically as possible the subject matter to be searched. Define any terms that may have special meaning. Give examples or relevant citations, authors, or keywords, if known.

You may include a copy of the broadest and or relevant claim(s).

Please search:

1) hybridiz? and light? and amplif? and nucleic (w) acid#

2) Rodney M. Richards

Please search

CAS, BIOSIS, and MEDLINE

STAFF USE ONLY

COMPLETED 3-17-89

SEARCHER

ONLINE TIME TOTAL TIME
(in minutes)

NO. OF DATABASES

SYSTEMS

____ CAS ONLINE
____ DARC/QUESTEL
__✓__ DIALOG
____ SDC
____ OTHER

220108
3/20/89

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s hybridiz?(p)ligat?(p)amplif?(p)nucleic(w)acid#
          1282 HYBRIDIZ?
          2385 LIGAT?
        102476 AMPLIF?
          2345 NUCLEIC
        208635 ACID#
L1             0 HYBRIDIZ?(P)LIGAT?(P)AMPLIF?(P)NUCLEIC(W)ACID#

=> s hybridiz?(p)ligat?(p)nucleic(w)acid#
          1282 HYBRIDIZ?
          2385 LIGAT?
          2345 NUCLEIC
        208635 ACID#
L2            12 HYBRIDIZ?(P)LIGAT?(P)NUCLEIC(W)ACID#

=> s l2 and amplif?
        102476 AMPLIF?
L3             5 L2 AND AMPLIF?

=> d 1-5

1.  4,785,086, Nov. 15, 1988, Test for Campylobacter; Ayoub Rashtchian, et
al., 536*27; 435*6, 172.3, 320; 935*72, 78

2.  4,760,025, Jul. 26, 1988, Modified enzymes and methods for making same;
David A. Estell, et al., 435*222; 252*547; 435*91, 172.1, 172.3, 221; 935*10,
14

3.  4,749,647, Jun. 7, 1988, Polymerization-induced separation assay using
recognition pairs; Elaine K. Thomas, et al., 435*6, 7; 436*501, 504, 538,


539, 548, 827; 525*904; 526*238.1; 527*202; 536*27; 935*78

4.  4,661,450, Apr. 28, 1987, Molecular cloning of RNA using RNA ligase and
synthetic oligonucleotides; Tomas Kempe, et al., 435*172.3, 68, 91, 320;
536*27

5.  4,518,690, May 21, 1985, DNA promoter sequence of Avian tumor virus and
use thereof for enhanced gene expression in E. coli; Ramareddy V. Guntaka,
435*71, 68, 70, 172.3, 252.33, 320; 536*27; 935*8, 32, 73

=> d l3 kwic 1-5

US PAT NO:     4,785,086                                  L3: 1 of 5

SUMMARY:

BSUM(19)
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Our . . . there is not just one, but a number, of specific probes provides the added advantage of increased sensitivity and signal amplification; the larger the number of different probes used, the greater the sensitivity of the assay. This is because when several. . .

DETDESC:

DETD(4)

A . . . in Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory. One such vector is pUN121 [Nilsson et al. (1983) Nucleic Acids Research 11:8019-8030]. The DNA from C. jejuni N941 (ATCC 39983) is digested with restriction endonuclease HindIII, creating fragments of different sizes. These fragments are then ligated into the HindIII site of a suitable vector using the general method described in Cohen et al.,

DETD(4)
(1973) Proc. Nat'l Acad. Sci. 70:3240. This ligation mix is then used to transform E. coli HB101. The transformants are selected on the basis of a suitable marker.. . . et al. (1982) cited above; and the DNA is probed with nick-translated E. coli DNA. The plasmids which do not hybridize to E. coli DNA (about 95%) are selected and purified by CsCl-ethidium bromide gradients. The insert DNA is purified from. . .

US PAT NO: 4,760,025 L3: 2 of 5

DETDESC:

DETD(50)

Cells . . . grown 10-12 hours at 37.degree. C. and the filters transferred to fresh plates containing LB and 150 .mu.g/ml spectinomycin to amplify the plasmid pool.

DETD(50)

DETDESC:

DETD(107)

The primer was hybridized to M-13 mp9 SUBT as modified from Norris et al., 1983, "Nucleic Acids Res." 11, 5103-5112 by combining 5 .mu.L of the labelled mutagenesis primer (.sup..about. 3 .mu.M), .sup..about. 1 .mu.g M-13 mp9. . . .mu.L of 10 mM ATP, 1 .mu.L ligase (4 units) and 1 .mu.L Klenow (5 units). The primer extension and ligation reaction (total volume 25 .mu.l) proceeded 2 hours at 14.degree. C. The Klenow and ligase were inactivated by heating to. . .

US PAT NO: 4,749,647 L3: 3 of 5

US PAT NO: 4,749,647 L3: 3 of 5

SUMMARY:

BSUM(20)

Nucleic . . . by in situ hybridization, while fine structural changes are detected by Southern blotting techniques. It is also possible to demonstrate amplification of genetic information, either at the RNA level or the DNA level, using nucleic acid hybridization. Some or all of. . .

DETDESC:

DETD(54)

It . . . by a first component is absorbed by a second component, which emits at a different wavelength), etc. Because of the amplification

DETD(54)
achieved by physical transfer and concentration of the components of the signal-generating system in the microenvironment of the polymer, the. . .

DETDESC:

DETD(76)

The . . . sequence in any of several ways. For example, the analyte detecting sequence can be (1) tailed with terminal transferase; (2) ligated to a preformed polynucleotide; (3) cloned and replicated in various single- or double-stranded vectors, for example, M13 or pBR322; (4) extended by chemical synthesis; (5) extended by the addition of a non-nucleic acid tail; (6) labeled by nick translation using E. coli DNA polymerase I containing trace amounts of DNase I; (7) extended. . . such as polynucleotide phosphorylase; (9) extended by a template-dependent RNA polymerase such as QB replicase or SP6 polymerase; or (10) hybridized

DETD(76)
to a pre-formed polynucleotide or polynucleotide/polymer conjugate having a sequence which is complementary to part of the analyte detecting sequence. .
.

DETDESC:

DETD(81)

Among the methods of synthesizing probes, it is possible to ligate or hybridize the tail of an analyte detecting sequence to a complementary labeled preformed polynucleotide or to chemically introduce a non-nucleic acid tail. The advantage of these methods is that a variety of different analyte detecting sequences can be labeled by hybridization to a single complementary pre-formed polynucleotide. The preformed polynucleotide can be labeled during or after synthesis by any of the methods described below. For example, an analyte detecting sequence having a poly(C) tail can be

DETD(81)
hybridized to a labeled pre-formed polynucleotide containing a poly(G) tail. To prevent dissociation of the hybridized sequences, the probe can be cross-linked, for example, by irradiation at 365 nm in the presence of psoralen (Hochkeppel et. . .

DETDESC:

DETD(136)

Analyte . . . is that one has as many as 7,000 nucleotides (in M13) which can potentially be labeled. This affords a tremendous amplification of signal which can be especially important when the analyte is present in low concentration.

US PAT NO: 4,661,450 L3: 4 of 5

US PAT NO: 4,661,450 L3: 4 of 5

SUMMARY:

BSUM(63)

One . . . Several modifications of this procedure which eliminate the need for S1 nuclease digestion have been reported (Land, et al.; 1981, Nucleic Acids Res. 9: 2251-2266). More recently, Okayama and Berg (1982, Molecular and Cellular Biology 2(2): 161-17Ø) reported a method for inserting. . . molecules into DNA cloning vectors which have been modified by oligo(dT) tailing, so that the 3'-poly(A) tail of the mRNA hybridizes to the oligo(dT) tail of the vector. After cDNA synthesis, the free ends of the plasmid and vector are modified to allow hybridization, and finally, ligation.

BSUM(63)

SUMMARY:

BSUM(7Ø)

RNA . . . the oligonucleotide sequences. For a review of T4 RNA ligase properties and activity, see Gumport and Uhlenbeck, 198Ø, in "Gene Amplification and Analysis", Vol. II: Analysis of Nucleic Acid Structure by Enzymatic Methods, Chirikjian and Papas, eds. Elsevier North Holland, Inc.

DETDESC:

DETD(112)

The . . . removal of the 5'-cap from the .beta.-globin mRNA. (Lockard, et al., 1981, pages 229-251. In, Chirikjian & Papas, eds., Gene

DETD(112)
Amplification and Analysis, Vol. 2). TAP hydrolyzes the pyrophosphate bonds of the cap structure to yield a mixture of mRNA molecules. . .

US PAT NO: 4,518,69Ø L3: 5 of 5

DETDESC:

DETD(6Ø)

Ligation of viral DNA fragments to pBR322 DNA. Five ng avian tumor virus supercoiled DNA was mixed with 2ØØ ng pBR322. . . to 4 units restriction endonuclease Hind III (BRL, Bethesda, Md.) under the conditions specified by the supplier. Following digestion, the nucleic acids were deproteinized and precipitated by ethanol. The precipitates were collected by centrifugation and resuspended directly in 1Ø-2Ø .mu.l ligase buffer (2Ø mM Tris HCl, pH 7.6, 6.6 mM MgCl.sub.2, 1Ø mM Dithiothreitol and Ø.1 mM ATP).

DETD(6Ø)
Ligation was carried out at 15.degree. C. for 16-2Ø hours with Ø.5 to 1.Ø unit of T.sub.4 DNA ligase (Bolivar, F.,. . . on agarose gel followed by blotting according to the procedure of Southern, E. M., J. Mol. Biol. 98:5Ø3-517 (1975) and nucleic acid hybridization to detect viral DNA using .sup.32 P-labled cDNA.

DETDESC:

DETD(62)

Preparation . . . L broth. (Bolivar, F., Rodriguez, R. L., Betlach, M. C. and Boyer, H. W., Gene, 2:7[illegible]3 (1977)) and plasmid DNA amplified with 1ØØ .mu.g/ml chloroamphenicol, was prepared by the SDS-NaCl method. (Clewell, D. B., J. Bacteriol., 11Ø:667-676 (1972)). The DNA was. . .

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 => d l2 1=12
'1=12' IS NOT VALID HERE

=> d l2 1-12
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1. 4,797,355, Jan. 10, 1989, Methods for attaching polynucleotides to supports; Yitzhak Stabinsky, 435*6; 436*501; 935*77, 78

2. 4,785,086, Nov. 15, 1988, Test for Campylobacter; Ayoub Rashtchian, et al., 536*27; 435*6, 172.3, 320; 935*72, 78

3. 4,760,025, Jul. 26, 1988, Modified enzymes and methods for making same; David A. Estell, et al., 435*222; 252*547; 435*91, 172.1, 172.3, 221; 935*10, 14

4. 4,757,013, Jul. 12, 1988, Cloning vehicles for polypeptide expression in microbial hosts; Masayori Inouye, et al., 435*172.3, 68, 70, 252.33, 320; 935*27, 40, 41, 43, 48, 60, 73

5. 4,749,647, Jun. 7, 1988, Polymerization-induced separation assay using recognition pairs; Elaine K. Thomas, et al., 435*6, 7; 436*501, 504, 538, 539, 548, 827; 525*904; 526*238.1; 527*202; 536*27; 935*78

6. 4,666,836, May 19, 1987, Novel cloning vehicles for polypeptide expression in microbial hosts; Masayori Inouye, et al., 435*68, 172.3, 252.33, 320; 935*29, 41, 43

7. 4,661,450, Apr. 28, 1987, Molecular cloning of RNA using RNA ligase and synthetic oligonucleotides; Tomas Kempe, et al., 435*172.3, 68, 91, 320; 536*27

8. 4,657,857, Apr. 14, 1987, Yeast of the genus kluyveromyces modified for the expression of preprothaumatin or its various allelic and modified forms or their maturation forms, and the proteins obtained by that expression; Luppo Edens, et al., 435*68, 172.3, 255, 320; 544*132; 548*165, 221, 251, 329; 935*11, 28, 56, 69

9. 4,643,969, Feb. 17, 1987, Novel cloning vehicles for polypeptide expression in microbial hosts; Masayori Inouye, et al., 435*68, 172.3, 320; 935*6, 29, 41, 48, 56, 72

10. 4,631,259, Dec. 23, 1986, Transposon in cloning DNA; Don B. Clewell, et al., 435*172.3, 68, 320; 935*23, 38, 56, 73

11. 4,624,926, Nov. 25, 1986, Novel cloning vehicles for polypeptide expression in microbial hosts; Masayori Inouye, et al., 435*252.33, 172.3; 935*29, 41, 48, 73

12. 4,518,690, May 21, 1985, DNA promoter sequence of Avian tumor virus and use thereof for enhanced gene expression in E. coli; Ramareddy V. Guntaka, 435*71, 68, 70, 172.3, 252.33, 320; 536*27; 935*8, 32, 73

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=> s ligat?(p)amplif?(p)nucleic(w)acid#
          2385 LIGAT?
        102476 AMPLIF?
          2345 NUCLEIC
        208635 ACID#
L4            10 LIGAT?(P)AMPLIF?(P)NUCLEIC(W)ACID#

=> d 1-10
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1. 4,784,949, Nov. 15, 1988, Universal dominant selectable marker cassette; David H. Gelfand, et al., 435*68, 172.3, 252.31, 252.33, 252.34, 254, 255, 320; 536*27; 935*14, 27, 28, 29, 47

2. 4,752,585, Jun. 21, 1988, Oxidation-resistant muteins; Kirston E. Koths,

et al., 435*252.33, 172.3, 252.3, 256, 320; 530*351; 536*27; 935*10, 111

3. 4,711,845, Dec. 8, 1987, Portable temperature-sensitive control cassette; David H. Gelfand, et al., 435*68, 91, 172.3, 252.3, 252.33, 317.1; 536*27; 935*11, 29, 41, 43, 45, 73 [IMAGE AVAILABLE]

4. 4,703,009, Oct. 27, 1987, RDNA cloning vector pVE1, deletion and hybrid mutants and recombinant derivatives thereof products and processes; Tanya MacNeil, et al., 435*172.3, 68, 91, 243, 252.35, 320, 886, 906; 935*29, 72, 73, 74, 75 [IMAGE AVAILABLE]

5. 4,677,064, Jun. 30, 1987, Human tumor necrosis factor; David F. Mark, et al., 435*68; 424*85.1, 88; 435*172.3, 252.3, 320; 514*8; 530*350, 351, 395, 808; 536*27

6. 4,677,063, Jun. 30, 1987, Human tumor necrosis factor; David F. Mark, et al., 435*68; 424*85.1, 88; 435*172.3, 240.2, 252.3, 252.33, 320; 514*8, 12;

530*350, 351, 395, 808; 536*27

7. 4,659,805, Apr. 21, 1987, Recombinant alveolar surfactant protein; James W. Schilling, Jr., et al., 530*350, 324

8. 4,631,191, Dec. 23, 1986, Methods and compositions useful in preventing equine influenza; Beverly Dale, et al., 424*88, 89; 530*324, 325, 326, 806, 811

9. 4,508,826, Apr. 2, 1985, Bacteriophage DNA cloning vector TG1 and microorganisms containing TG1; Forrest Foor, et al., 435*235, 172.2, 239, 252.3, 252.35, 320; 935*31, 75

10. 4,460,689, Jul. 17, 1984, DNA Cloning vector TG1, derivatives, and processes of making; Forrest Foor, et al., 435*172.3, 68, 235, 239, 252.35, 320, 886; 935*9, 12, 23, 31, 41, 73, 75

=> s l4 and hybridiz?
          1282 HYBRIDIZ?
L5   10 L4 AND HYBRIDIZ?

=> d kwic 1-10

US PAT NO: 4,784,949 L5: 1 of 10

DETDESC:

DETD(45)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by transforming E. coli strain MM294 obtained from E. coli Genetic Stock Center, CGSC #6135, or other suitable host with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending

DETD(45)
on the mode. . . prepare[redacted]ccording to the method of [redacted]lewell, D. B., et al, Proc Natl Acad Sci (U.S.A.) (1969) 62:1159, following chloramphenicol amplification (Clewell, D. B., J Bacteriol (1972) 110:667). The isolated

DNA is analyzed by restriction and/or sequenced by the dideoxy method of Sanger, F. et al, Proc Natl Acad Sci (U.S.A.) (1977) 74:5463 as further desribed by Messing, et al, Nucleic Acids Res (1981) 9:309, or by the method of Maxam, et al, Methods in Enzymology (1980) 65:499.

DETDESC:

DETD(54)

Before hybridization with probe, the nitrocellulose filters were prehybridized for 3 hours to overnight at 42.degree. C. in 50% formamide, 5.times.SSC, 1/20. . .

DETD(54)

DETDESC:

DETD(55)

The filters were hybridized with 106 cpm of (usually) .sup.32 P-labelled, nick-translated DNA probe in a solution of 50% formamide, 5.times.SSC, 1/20 P/Pi, 0.1%. . .

DETDESC:

DETD(56)

The hybridized filters were washed three times in 2.times.SSC, 0.1% SDS at room temperature, dried and exposed to x-ray film.

US PAT NO: 4,752,585 L5: 2 of 10

DRAWING DESC:

DRWD(30)

To . . . of the plaques will consist of phage containing the mutated form; 50% will have the original sequence. The plaques are hybridized with kinased synthetic primer under stringency conditions which permit hybridization only with the desired sequence, which will form a perfect match with the probe. Hybridizing plaques are then picked and cultured, and the DNA is recovered.

DRAWING DESC:

DRWD(67)

DRWD(67)

Most of the techniques which are used to transform cells, construct vectors, effect hybridization with probe, and the like are widely practiced in the art, and most practitioners are familiar with the standard resource. . .

DRAWING DESC:

DRWD(85)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by first transforming E. coli strain MM294 obtained from E. coli Genetic Stock Center, CGSC #6135, or other suitable host, with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . to the method of Clewell, D. B., et al., Proc. Natl. Acad. Sci. (USA) (1969) 62: 1159, optionally following

chloramphenicol amplification (Clewell, D. B., J. Bacteriol. (1972) 110:

DRWD(85)

d 15 kwic 1-10

US PAT NO: 4,784,949 L5: 1 of 10

DETDESC:

DETD(45)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by transforming E. coli strain MM294 obtained from E. coli Genetic Stock Center, CGSC #6135, or other suitable host with the

DETD(45)
ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . prepared according to the method of Clewell, D. B., et al, Proc Natl Acad Sci (U.S.A.) (1969) 62:1159, following chloramphenicol amplification (Clewell, D. B., J Bacteriol (1972) 110:667). The isolated DNA is analyzed by restriction and/or sequenced by the dideoxy method of Sanger, F. et al, Proc Natl Acad Sci (U.S.A.) (1977) 74:5463 as further desribed by Messing, et al, Nucleic Acids Res (1981) 9:309, or by the method of Maxam, et al, Methods in Enzymology (1980) 65:499.

DETDESC:

DETD(54)

Before hybridization with probe, the nitrocellulose filters were prehybridized for 3 hours to overnight at 42.degree. C. in 50% formamide,

DETD(54)
5.times.SSC, 1/20. . .

DETDESC:

DETD(55)

The filters were hybridized with 106 cpm of (usually) .sup.32 P-labelled, nick-translated DNA probe in a solution of 50% formamide, 5.times.SSC, 1/20 P/Pi, 0.1%. . .

DETDESC:

DETD(56)

The hybridized filters were washed three times in 2.times.SSC, 0.1% SDS at room temperature, dried and exposed to x-ray film.

DETD(56)

US PAT NO: 4,752,585 L5: 2 of 10

DRAWING DESC:

DRWD(30)

To . . . of the plaques will consist of phage containing the mutated form; 50% will have the original sequence. The plaques are hybridized with kinased synthetic primer under stringency conditions which permit

hybridization only with the desired sequence, which will form a perfect match with the probe. Hybridizing plaques are then picked and cultured, and the DNA is recovered.

DRAWING DESC:

DRWD(67)

Most of the techniques which are used to transform cells, construct vectors, effect hybridization with probe, and the like are widely practiced in the art, and most practitioners are familiar with the standard resource. . .

DRAWING DESC:

DRWD(85)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by first transforming E. coli strain MM294 obtained from E. coli Genetic Stock Center, CGSC #6135, or other suitable host, with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . to the method of Clewell, D. B., et al., Proc. Natl. Acad. Sci. (USA) (1969) 62: 1159, optionally following

DRWD(85)

chloramphenicol amplification (Clewell, D. B., J. Bacteriol. (1972) 110: 667). The isolated DNA is analyzed by restriction enzyme analysis and/or the DNA. . . of Sanger, F., et al., Proc. Natl. Acad. Sci. (USA) (1977) 74: 5463 as further described by Messing, et al., Nucleic Acids Res. (1981) 9: 309, or by the method of Maxam, et al., Methods in Enzymology (1980) 65: 499.

DETDESC:

DETD(28)

D.1 . . . oligonucleotide 5'-GATGATGCTCTGAGAAAAGGTAATC-3' was kinased under standard conditions for use as primer and probe. Ten pmoles of the kinased primer was hybridized to 2.6 .mu.g of single-stranded (ss) M13-IL-2 DNA in 15 .mu.l of a mixture containing 100 mM NaCl, 20 mM. . . agar plates, and incubated overnight to obtain phage plaques. The plaques

DETD(28)

were probed using kinased primer using standard prehybridization and hybridization conditions at high stringency (42.degree. C. for eight hours). A plaque which hybridized to primer was picked. This plaque was designated M13-LW46, and contains the coding sequence for des-ala.sub.1 ser.sub.125 IL-2.

DETDESC:

DETD(30)

One of the mutagenized M13-LW46 plaques which hybridized with probe was designated SDL23, picked, cultured, and used to prepare expression vector pSY3001.

DETDESC:

DETD(138)

A . . . oligonucleotide 5'-CCATCTATGAGGCGCTGCAGAACA-3' was kinased under standard conditions for use as primer and probe. Ten pmoles of the kinased primer was hybridized to 2.6 .mu.g of single-stranded (SS)

M13-SY2501 in 15 .mu.l of a mixture containing 100 mM NaCl, 20 mM Tris-HCl,. . . MD on July 13, 1984 under ATCC No. 39,768. The plaques were probed using kinased primer using standard prehybridization and hybridization conditions at high stringency (60.degree. C. for two hours). A plaque which hybridized to primer was selected. This plaque was designated M13-DM101 and contains the coding sequence for ser.sub.17 ala.sub.62 IFN-.beta..

US PAT NO: 4,711,845 [IMAGE AVAILABLE] L5: 3 of 10

DETDESC:

DETD(25)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by transforming E. coli strain MM294 obtained from E. coli Genetic Stock Center, CGSC #6135, or other suitable host with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . . prepared according to the method of Clewell, D. B., et al, Proc Natl Acad Sci (1969) 62: 1159, following chloramphenicol amplification (Clewell, D. B., J Bacteriol (1972) 110: 667) and analyzed by restriction and/or sequenced by the method of Messing, et al, Nucleic Acids Res (1981) 9: 309, or by the method of Maxam, et al, Methods in Enzymology (1980) 65: 499.

DETDESC:

DETD(81)

The . . . amino acids prior to a termination codon was synthesized using the triester method of Matteucci, et al (supra); kinased and hybridized to the complementary synthetic fragment as described in paragraph F.2 in connection with pCS3DT synthesis. One pmole double-stranded oligonucleotide was. . .

US PAT NO: 4,703,009 [IMAGE AVAILABLE] L5: 4 of 10

ABSTRACT:
Novel plasmid pVE1, deletion mutants thereof, recombinant derivatives thereof, which is the same as the genome or nucleic acid of such plasmids and derivatives of such genome, which are useful as recombinant DNA cloning vectors into host organisms, such. . . an autonomous element; 2. to serve as promoters for increasing expression of endogenous or foreign genes wherein said promoters are ligated to such genes or otherwise serve

US PAT NO: 4,703,009 [IMAGE AVAILABLE] L5: 4 of 10
as promoters; and 3. to serve as regulatory elements for achieving control over endogenous and foreign gene expression; as cloning vectors, pVE1 its deletion mutants, and other derivatives serve for the amplification and transfer of DNA sequences (genes) coding for useful functions, such modified cloning vectors are introduced into the recipient organism. . .

DETDESC:

DETD(57)

DNA . . . et al., 1981, supra). This fragment is tailed with approximately 10 to 15 deoxycytidine nucleotide residues. The fragment is then hybridized with the tailed vector, and the mixture used to transform S. lividans protoplasts to Thio.sup.r and screened for neomycin sensitivity. . .

US PAT NO: 4,677,064 L5: 5 of 10

DETDESC:

DETD(28)

mRNA . . . affect TNF production in this translation system ("hybrid arrest"). This criterion can be further refined by radioautography of mRNA gradients hybridized to the kinased probes where the desired TNF encoding mRNA has previously been identified by translation of the fractions in. . . only to TNF-encoding mRNA, these "hydrid arrest" experiments were repeated using eight pairs of 14-mers. One pair was successful in hybridizing the mRNA specifically.

DETDESC:

DETD(29)

Once . . . identified, it was used to probe a cDNA library formed from the mRNA fraction encoding the desired TNF. Twenty-eight successful hybridizing colonies were picked, plasmid DNA isolated, and several inserts sequenced. A plasmid preparation containing the entire coding sequence, designated pE4,. . .

DETDESC:

DETD(49)

cDNA or genomic libraries are screened using the colony hybridization procedure. Each microtiter plate is replicated onto duplicate nitrocellulose filter papers (S & S type BA-85) and colonies are allowed. . . 2 hr. The duplicate filters are prehybridized at 42.degree. C. for 6-8 hr with 10 ml per filter of DNA hybridization buffer (5.times.SSC, pH 7.0

DETD(49)
5.times.Denhardt's solution (polyvinylpyrrolidine, plus Ficoll and bovine serum albumin; 1.times.=0.02% of each), 50 mM sodium phosphate. . .

DETDESC:

DETD(50)

The samples are hybridized with kinased probe under conditions which depend on the stringency desired. Typical moderately stringent conditions employ a temperature of 42.degree. C. for 24-36 hr with 1-5 ml/filter of DNA hybridization buffer containing probe. For higher stringencies high temperatures and shorter times are employed. The filters are washed four times for. . .

DETDESC:

DETD(59)

Theoretically, . . . the phage having, as a single strand, the mutated form; 50% will have the original sequence. The resulting plaques are hybridized with kinased synthetic primer at a temperature which permits hybridization of an exact match, but at which the mismatches with the original strand are sufficient to prevent hybridization. Plaques which hybridize with the probe are then picked, cultured, and the DNA recovered. Details of site specific mutation procedures are described below. . .

DETDESC:

DETD(61)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by first transforming E. coli strain MM294

DETD(61)
obtained from E. coli Genetic Stock Center, CGSC #6135, or other suitable host with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . according to the method of Clewell, D. B., et al, Proc Natl Acad Sci (USA) (1969) 62:1159, optionally following chloramphenicol amplification (Clewell, D. B., J Bacteriol (1972) 110:667). The isolated DNA is analyzed by restriction and/or sequenced by the dideoxy method of Sanger, F., et al, Proc Natl Acad Sci (USA) (1977) 74:5463 as further described by Messing, et al, Nucleic Acids Res (1981) 9:309, or by the method of Maxam, et al, Methods in Enzymology (1980) 65:499.

DETDESC:

DETD(90)

Oligomers . . . the pool having the sequence ##STR2## was inactive. The

DETD(90)
specificity of this oligomer pool was further determined using "dot blot" hybridization with enriched mRNA prepared as above from both induced and uninduced HL-60 cells, as well as the corresponding mRNA fraction obtained from cells known to be producers of lymphotoxin. This pool hybridized well to the induced mRNA, but failed to hybridize with the corresponding fractions from the uninduced or lymphotoxin producing cells. However, Northern blots using the kinased pool as probe demonstrated that it contained sequences which cross hybridize with the 18S (ribosomal) RNA fraction and to pBR322 DNA.

DETDESC:

DETD(91)

The . . . RNA, and pBR322 DNA confirmed the specificity of the foregoing 14-mer pair and the inability of the remaining pairs to hybridize to the

DETD(91)
desired messenger.

DETDESC:

DETD(93)

The cDNA library prepared above was probed with the 14-mer pair identified in D.2.c. Twenty-eight colonies which hybridized with probe were picked, cultured, and the plasmid DNA isolated. Plasmids containing inserts of sufficient length to encode the entire. . .

DETDESC:

DETD(94)

The . . . for protein, which is then tested in the .sup.35 S version of

DETD(94)
the L-929 cytotoxic assay. The results for several hybridizing clones, designated E2-E4, E6 and E8 are shown below: ##TBL1## (A+ and B+ are controls using enriched mRNA as obtained. . .

DETDESC:

DETD(105)

Ten picomoles of the oligonucleotide were hybridized to 2.6 .mu.g of ss clone 4.1 DNA in 15 .mu.l of a mixture containing 100 mM NaCl, 20 mM. . .

DETDESC:

DETD(106)

Plates . . . in 2.times.SSC, dried and then baked in a vacuum oven at

DETD(106)
80.degree. C. for 2 hr. The duplicate filters were pre-hybridized at 42.degree. C. for 4 hr with 10 ml per filter of DNA hybridization buffer (5.times. SSC, pH 7.0, 4.times.Denhardts solution (polyvinylpyrrolidine, ficoll and bovin serum albumin, 1.times.=0.02% of each), 0.1% SDS, 50 mM. . . of denatured salmon sperm DNA. .sup.32 P-labeled probes were prepared by kinasing the primer with labeled ATP. The filters were hybridized to 5.times.10.sup.6 cpm/ml of .sup.32 P-labeled primer in 1-5 ml per filter of DNA hybridization buffer at 64.degree. C. for 8 hr.

DETDESC:

DETD(108)

Since . . . designed to create a new HindIII restriction site in the mutagenized clones, RF-DNA from a number of the clones which hybridized with the primer were digested with this restriction enzyme. One of the

DETD(108)
mutagenized clone 4.1 plaques which has a new. . .

DETDESC:

DETD(123)

The . . . in D.2.d above, contains the SV40 promoter in operable linkage to the TNF coding sequence. All of the 28 positively hybridizing colonies would be expected to contain this linkage, including, specifically pE4 and pB11, and are thus capable of expression in. . .

DETDESC:

DETD(139)

Ten picomoles of the oligonucleotide were hybridized to 2.6 .mu.g of ss

DETD(139)
clone M13-AW711 DNA in 15 .mu.l of a mixture containing 100 mM NaCl, 20 mM. . .

DETDESC:

DETD(140)

Plates . . . in 2.times.SSC, dried and then baked in a vacuum oven at 80.degree. C. for 2 hr. The duplicate filters were pre-hybridized at 42.degree. C. for 4 hr with 10 ml per filter of DNA hybridization buffer (5.times.SSC, pH 7.0, 4.times.Denhardts solution (polyvinylpyrrolidine, ficoll and bovin serum albumin, 1.times.=0.02% of each), 0.1% SDS, 50 mM sodium. . . of denatured salmon sperm DNA. .sup.32 P-labeled probes were prepared by kinasing the primer with labeled ATP. The filters were hybridized to 5.times.10.sup.6 cpm/ml of .sup.32 P-labeled primer in 1-5

per filter of DNA hybridization buffer at 64.degree. C. for 6 hr.

DETD(140)

US PAT NO: 4,677,063 L5: 6 of 10

DETDESC:

DETD(30)

mRNA . . . affect TNF production in this translation system ("hybrid arrest"). This criterion can be further refined by radioautography of mRNA gradients hybridized to the kinased probes where the desired TNF encoding mRNA has previously been identified by translation of the fractions in. . . it was possible to show by Northern blots that even this mixture of sixteen oligomers was not sufficiently specific to hybridize only to TNF-encoding mRNA, these "hybrid arrest" experiments were repeated using eight pairs of 14-mers. One pair was successful in hybridizing the mRNA specifically.

DETD(30)

DETDESC:

DETD(31)

Once . . . identified, it was used to probe a cDNA library formed from the mRNA fraction encoding the desired TNF. Twenty-eight successful hybridizing colonies were picked, plasmid DNA isolated, and several inserts sequenced. A plasmid preparation containing the entire coding sequence, designated pE4,. . .

DETDESC:

DETD(56)

cDNA or genomic libraries are screened using the colony hybridization

DETD(56)
procedure. Each microtiter plate is replicated onto duplicate nitrocellulose filter papers (S & S type BA-85) and colonies are allowed. . . 2 hr. The duplicate filters are prehybridized at 42.degree. C. for 6-8 hr with 10 ml per filter of DNA hybridization buffer (5.times.SSC, pH 7.0 5.times.Denhardt's solution (polyvinylpyrrolidine, plus Ficoll and bovine serum albumin; 1.times.=0.02% of each), 50 mM sodium phosphate. . .

DETDESC:

DETD(57)

The samples are hybridized with kinased probe under conditions which depend on the stringency desired. Typical moderately stringent conditions employ a temperature of 42.degree. C. for 24-36 hr with 1-5 ml/filter of DNA hybridization buffer containing probe. For higher stringencies high temperatures and shorter times are employed. The filters are washed four

DETD(57)
times for. . .

DETDESC:

DETD(66)

Theoretically, . . . the phage having, as a single strand, the mutated form; 50% will have the original sequence. The resulting plaques are hybridized with kinased synthetic primer at a temperature which permits hybridization of an exact match, but at which the mismatches with the original strand are sufficient to prevent hybridization. Plaques which hybridize with the probe are then picked, cultured, and the DNA recovered. Details of site specific mutation procedures are described below. . .

DETD(66)

DETDESC:

DETD(68)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by first transforming E. coli strain MM294 obtained from E. coli Genetic Stock Center, CGSC #6135, or other suitable host with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . to the method of Clewell, D. B., et al, Proc Natl Acad Sci (USA) (1969) 62: 1159, optionally following chloramphenicol amplification (Clewell, D. B., J Bacteriol (1972) 110: 667). The isolated DNA is analyzed by restriction and/or sequenced by the dideoxy. . . of Sanger, F., et al, Proc Natl Acad Sci (USA) (1977) 74: 5463 as further described by Messing, et al, Nucleic Acids Res (1981)

DETD(68)
9: 309, or by the method of Maxam, et al, Methods in Enzymology (1980) 65: 499.

DETDESC:

DETD(97)

Oligomers . . . the pool having the sequence ##STR2## was inactive. The specificity of this oligomer pool was further determined using "dot blot" hybridization with enriched mRNA prepared as above from both induced and uninduced HL-60 cells, as well as the corresponding mRNA fraction obtained from cells known to be producers of lymphotoxin. This pool hybridized well to the induced mRNA, but failed to hybridize with the corresponding fractions from the uninduced or lymphotoxin producing cells. However, Northern blots usng the kinased pool as probe demonstrated that it contained sequences which cross hybridize with the 18S (ribosomal) RNA fraction and

DETD(97)
to pBR322 DNA.

DETDESC:

DETD(98)

The . . . RNA, and pBR322 DNA confirmed the specificity of the foregoing 14-mer pair and the inability of the remaining pairs to hybridize to the desired messenger.

DETDESC:

DETD(100)

The cDNA library prepared a[illegible]ve was probed with the 14[illegible]r pair identified in D.2.c. Twenty-eight colo[illegible]es which hybridized with p[illegible]obe were picked,

DETD(100)
cultured, and the plasmid DNA isolated. Plasmids containing inserts of sufficient length to encode the entire. . .

DETDESC:

DETD(101)

The . . . for protein, which is then tested in the .sup.35 S version of the L-929 cytotoxic assay. The results for several hybridizing clones, designated E2-E4, E6 and E8 are shown below: ##TBL1## (A+ and B+ are controls using enriched mRNA as obtained. . .

DETDESC:

DETD(112)

DETD(112)
Ten picomoles of the oligonucleotide were hybridized to 2.6 .mu.g of ss clone 4.1 DNA in 15 .mu.l of a mixture containing 100 mM NaCl, 20 mM. . .

DETDESC:

DETD(113)

Plates . . . a 2.times.SSC, dried and then baked in a vacuum oven at 80.degree. C. for 2 hr. The duplicate filters were pre-hybridized at 42.degree. C. for 4 hr with 10 ml per filter of DNA hybridization buffer (5 .times.SSC, pH 7.0, 4.times.Denhardts solution (polyvinylpyrrolidine, ficoll and bovin serum albumin, 1.times.=0.02% of each), 0.1% SDS, 50 mM. . . of denatured salmon sperm DNA. .sup.32 P-labeled probes were prepared by kinasing the primer with labeled ATP. The filters were hybridized to 5.times.10.sup.6 cpm/ml of .sup.32 P-labeled primer in 1-5 ml per filter of DNA hybridization buffer at 64.degree. C. for 8 hr.

DETD(113)

DETDESC:

DETD(115)

Since . . . designed to create a new HindIII restriction site in the mutagenized clones, RF-DNA from a number of the clones which hybridized with the primer were digested with this restriction enzyme. One of the mutagenized clone 4.1 plaques which has a new. . .

DETDESC:

DETD(130)

The . . . in D.2.d above, contains the SV40 promoter in operable linkage to the TNF coding sequence. All of the 28 positively hybridizing colonies

DETD(130)
would be expected to contain this linkage, including, specifically pE4 and pB11, and are thus capable of expression in. . .

DETDESC:

DETD(144)

Ten picomoles of the oligonucleotide were hybridized to 2.6 .mu.g of ss clone M13-AW701 DNA in 15 .mu.l of a mixture containing 100 mM NaCl, 20 mM. . .

DETDESC:

DETD(145)

Plates . . . in 2.times.SSC, dried and then baked in a vacuum oven at

DETD(145)
80.degree. C. for 2 hr. The duplicate filters were pre-hybridized at 42.degree. C. for 4 hr with 10 ml per filter of DNA hybridization buffer (5.times.SSC, pH 7.0, 4.times.Denhardts solution (polyvinylpyrrolidine, ficoll and bovin serum albumin, 1x=0.02% of each), 0.1% SDS, 50 mM sodium. . . of denatured salmon sperm DNA. .sup.32 P-labeled probes were prepared by kinasing the primer with labeled ATP. The filters were hybridized to 5.times.10.sup.6 cpm/ml of .sup.32 P-labeled primer in 1-5 ml per filter of DNA hybridization buffer at 64.degree. C. for 8 hr.

US PAT NO: 4,659,805 L5: 7 of 10

DETDESC:

DETD(18)

The . . . cDNA from the library constructed in E. coli was probed using

DETD(18)
these oligonucleotide sets. False positives were minimized by requiring hybridization to more than one set. Successfully hybridizing clones were sequenced, and one was shown to contain the correct N-terminal sequence.

DETDESC:

DETD(42)

cDNA or genomic libraries are screened using the colony hybridization procedure. Each microtiter plate is replicated onto duplicate nitrocellulose filter papers (S & S type BA-85) and colonies are allowed. . .

DETDESC:

DETD(43)

DETD(43)
For nick-translated probe, the duplicate filters are prehybridized at 42.degree. C. for 16-18 hr with 10 ml per filter of DNA hybridization buffer (50% formamide (40% formamide if reduced stringency), 5.times.SSC, pH 7.0, 5.times. Denhardt's solution (polyvinylpyrrolidine, plus Ficoll and bovine serum. . .

DETDESC:

DETD(44)

Samples are hybridized with nick-translated DNA probes at 42.degree. C. for 12-36 hr for homologous species and 37.degree. C. for heterologous species contained in 5 ml of this same DNA hybridization buffer. The filters are washed two times [redacted] 30 min, each time at 50.[redacted]ree. C., in 0.2.times.SSC, 0.1% SDS for homologous species hybridizati[redacted], and at 50.degree. C. in 3.times.SSC, 0.1% SDS for heterologous species

DETD(44)
hybridization. Filters are air dried and autoradiographed for 1-3 days at 31 70.degree. C.

DETDESC:

DETD(45)

For . . . mer) oligonucleotide probes, the duplicate filters are prehybridized at 42.degree. C. for 2-8 hr with 10 ml per filter of oligo-hybridization buffer (6.times.SSC, 0.1% SDS, 1 mM EDTA, 5.times. Denhardt's, 0.05% sodium pyrophosphate and 50 .mu.g/ml denatured and sheared salmon sperm. . .

DETDESC:

DETD(46)

The samples are hybridized with kinased oligonucleotide probes of 15-30 nucleotides under conditions which depend on the composition of the oligonucleotide. Typical conditions employ a temperature of 30.degree.-42.degree. C. for 24-36 hr with 5 ml/filter of this same oligo-hybridization buffer containing probe. The filters are washed two times for 15 min at 23.degree. C., each time with 6.times.SSC, 0.1%. . . SDS and 50 mM sodium phosphate buffer at pH 7, then are washed once for 2 min at the calculated hybridization temperature with 6.times.SSC and 0.1% SDS, air dried, and are autoradiographed at -70.degree. C. for 2 to 3 days.

DETDESC:

DETD(58)

Theoretically, . . . the phage having, as a single strand, the mutated

DETD(58)
form; 50% will have the original sequence. The resulting plaques are hybridized with kinased synthetic primer at a temperature which permits hybridization of an exact match, but at which the mismatches with the original strand are sufficient to prevent hybridization. Plaques which hybridize with the probe are then picked, cultured, and the DNA recovered. Details of site specific mutation procedures are described below. . .

DETDESC:

DETD(60)

In the constructions set forth below, correct ligations for plasmid construction are confirmed by first transforming E. coli strain MC1061 obtained from Dr. M. Casadaban (Casadaban, M., et al, J Mol Biol (1980) 138: 179-207) or other suitable host with the ligation mixture. Successful

DETD(60)
transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . to the method of Clewell, D. B., et al, Proc Natl Acad Sci (USA) (1969) 62: 1159, optionally following chloramphenicol amplification (Clewell, D. B., J Bacteriol (1972) 110: 667). The isolated DNA is analyzed by restriction and/or sequenced by the dideoxy. . . of Sanger, F., et al, Proc Natl Acad Sci (USA) (1977) 74: 5463 as further described by Messing, et al, Nucleic Acids Res (1981) 9: 309, or by the method of Maxam, et al, Methods in Enzymology (1980) 65: 499.

DETDESC:

DETD(89)

D.3.b. Probe Hybridization

DETD(89)

DETDESC:

DETD(90)

Six . . . master filters were placed on agar plates containing 170 .mu./ml chloramphenicol for 18 hr. The colonies were then prepared for hybridization according to the method of Grunstein, M., and Hogness, D., Proc Natl Acad Sci (1975) 72: 3961-3972.

DETDESC:

DETD(92)

Duplicate filters were then hybridized with 5.times.10.sup.6 cpm of one of each .sup.32 P-labeled oligonucleotide probe (phosphorylated in accordance

DETD(92)
with Maniatis, T., et al, Molecular Cloning, (1982) Cold Spring Harbor Laboratories, pp. 122-123) per filter in 10 ml hybridization solution containing identical ingredients as the prehybridization solution. Filters with oligonucleotide probes a, b, and c were hybridized at 37.degree. C., 45.degree. C., and 41.degree. C., respectively. After 1 hr, the thermostat was lowered to 28.degree. C. for. . . and 37.degree. C. for probe b, after which the bath was allowed to equilibrate. Filters with probe c were not hybridized at a lower temperature. The filters were washed twice in 6.times.SSC, 0.1% SDS at room temperature for 15 min, then. . . Purified Genes (ed. D. D. Brown and C. F. Fox), Academic Press, NY, pp. 683-693; that is, T.sub.d =4(G+C)+2(A+T). The hybridized filters were then dried and autoradiographed on Kodak.RTM. XAR film with Dupont.RTM. Cronex intensifying screens until complete exposures were obtained.

DETDESC:

DETD(93)

A colony was considered positive if it hybridized in duplicate with all three oligonucleotide probes or with both probes a and b. Of several potential positive clones, one hybridized much more intensely with probes a and b as compared to the others. Sequencing of this clone demonstrated that it. . .

DETDESC:

DETD(96)

The . . . gel containing methylmercuric hydroxide by the method of Bailey, J. M. and Davidson, N., Anal Biochem (1976) 70: 75-85. mRNA hybridizing to probe was 1800-2000 nucleotides in length, clearly larger than the approximately 700 nucleotides needed for the coding sequence.

DETD(96)

DETDESC:

DETD(97)

The . . . DNA) at 42.degree. C. for 18 hr. 5.times.10.sup.5 cpm of .sup.32 P-labeled boiled DS-1 cDNA was added per ml fresh hybridization buffer and the filters were incubated in this buffer at 42.degree. C. for 16 hr. They were then washed in. . .

DETDESC:

DETD(102)

A . . . the nick-translation method of Rigby, P. W. J., et al, J Mol Biol (1977) 113: 237-251. Filters were prewashed in hybridization buffer

DETD(102)
(0.75M NaCl, 0.75M sodium nitrate, 40% formamide, 0.05% SDS, 0.02% bovine serum albumin, 0.02% Ficoll-400,000, 0.02% polyvinyl pyrollidone, 0.1%. . . .mu.g/ml denatured sheared salmon sperm DNA) at 42.degree. C. for 1 hr. 5.times.10.sup.5 cpm probe was added per ml fresh hybridization buffer and the filters were incubated in this buffer at 37.degree. C. for 16 hr. They were then washed in. . . sodium citrate and 0.1% SDS two times at 50.degree. C., and exposed for autoradiography overnight. Six potential clones containing sequences hybridizing to DS-1 cDNA were purified. The most strongly hybridizing clone, gHS-15, was characterized.

DETDESC:

DETD(103)

A 700 by EcoRI fragment from gHS-15 hybridized with the DS-1 probe and was chosen for sequence analysis. This EcoRI fragment was purified, inserted

DETD(103)
into M13mp9, sequenced and. . .

DETDESC:

DETD(124)

60,000 . . . plated on nitrocellulose filters which served as masters for two sets of replicas. The colony filters were then prepared for hybridization according to the method of Grunstein, M., and Hogness, D. (supra). The filters were baked for 2 hr at 80.degree.. . . DNA) at 37.degree. C. for 18 hr. One.times.10.sup.6 cpm of .sup.32 P-labeled Ds-1 probe was added per ml of fresh hybridization buffer then incubated for 16 hr at 37.degree. C. The filters were then washed in 0.45M NaCl and 0.045M sodium. . .

DETD(124)

DETDESC:

DETD(125)

One positively hybridizing clone, HS-6, was further analyzed by sequence determination; HS-6 harbors a 1.2 kb insert which can be released from the. . .

DETDESC:

DETD(128)

Approximately . . . plates containing the appropriate drug for the

selected vector. Successful tranformants are replicted onto duplicate sets or nitrocellulose filters for hybridization.

DETD(128)

DETDESC:

DETD(130)

One.times.10.sup.6 cpm of .sup.32 P-labeled HS-6 cDNA probe is added per ml of hybridization buffer and the filters incubated in this buffer for 16 hr at 42.degree. C. The filters are then washed in. . . citrate and 0.05% SDS two times each for 30 min at 50.degree. C., and exposed for autoradiography overnight. Clones which hybridize to HS-6 probe are further characterized by restriction digestion with EcoRI. Those clones over 1 kb should contain the entire. . .

US PAT NO: 4,631,191 L5: 8 of 10

US PAT NO: 4,631,191 L5: 8 of 10

DETDESC:

DETD(14)

The positively hybridizing cDNA clones were then sequenced and their identity as HA and NA genes was confirmed by the similarity of the. . .

DETDESC:

DETD(15)

The . . . probes are used to detect the EIV genome in biological samples such as blood or urine by their ability to hybridize to the target polynucleotide under stringent conditions.

DETD(15)

DETDESC:

DETD(42)

For nick-translated probe, the duplicate filters are prehybridized at 42.degree. C. for 16-18 hr with 10 ml per filter of DNA hybridization buffer (50% formamide (40% formamide if reduced stringency), 5x SSC, pH 7.0, 5x Denhardt's solution (polyvinylpyrrolidine, plus Ficoll and bovine. . .

DETDESC:

DETD(43)

Samples are hybridized with nick-translated DNA probes at 42.degree. C. for 12-36 hr for homologous species and 37.degree. C. for heterologous

DETD(43)
species contained in 5 ml of this same DNA hybridization buffer. The filters are washed two times for 30 min. each time at 50.degree. C., in 0.2x SSC, 0.1% SDS for homologous species hybridization, and at 50.degree. C. in 3x SSC, 0.1% SDS for heterologous species hybridization. Filters are air dried and autoradiographed for 1-3 days at -70.degree. .

DETDESC:

DETD(44)

For . . . mer) oligonucleotide probes, the duplicate filters are prehybridized at 42.degree. C. for 2-8 hr with 10 ml per filter of oligo-hybridization buffer (6x SSC, 0.1% SDS, 1 mM EDTA, 5x Denhardt's, 0.05% sodium pyrophosphate and 50 .mu.g/ml denatured and sheared salmon. . .

DETD(44)

DETDESC:

DETD(45)

The samples are hybridized with kinased oligonucleotide probes of 12-30 nucleotides under conditions which depend on the composition of the oligonucleotide. Typical conditions employ a temperature of 30.degree.-42.degree. C. for 24-36 hr with 5 ml/filter of this same oligo-hybridization buffer containing probe. The filters are washed two times for 15 min at 23.degree. C., each time with 6x SSC,. . . SDS and 50 mM sodium phosphate buffer at pH 7, then are washed once for 2 min at the calculated hybridization temperature with 6x SSC and 0.1% SDS, air dried, and are autoradiographed at -70.degree. C. for 2 to 3 days.

DETD(45)

DETDESC:

DETD(53)

Ligations for plasmid construction are confirmed by first transforming E. coli strain MC1061 obtained from Dr. M. Casadaban (Casadaban, M., et al, J Mol Biol (1980) 138: 179-207) or other suitable host with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode. . . to the method of Clewell, D. B., et al, Proc Natl Acad Sci (USA) (1969) 62: 1159, optionally following chloramphenicol amplification (Clewell, D. B., J Bacteriol (1972) 110: 667). The isolated DNA is analyzed by restriction and/or sequenced by the dideoxy. . . of Sanger, F., et al, Proc Natl Acad Sci (USA) (1977) 74: 5463 as further described by Messing, et al, Nucleic Acids Res (1981) 9: 309, or by the method of Maxam, et al, Methods in

DETD(53)
Enzymology (1980) 65: 499.

DETDESC:

DETD(56)

Theoretically, . . . the phage having, as a single strand, the mutated form; 50% will have the original sequence. The resulting plaques are hybridized with kinased synthetic primer and then washed at a temperature which permits hybrids of an exact match to remain, but at which the mismatches with the original strand are washed off. Plaques which remain hybridized to the probe at the stringent wash temperature are then picked, cultured, and the DNA recovered.

DETDESC:

DETD(58)

For . . . mer) oligonucleotide probes, the duplicate filters are

prehybridized at 42.degree. C. for 2-8 hr with 10 ml per filter of oligo-hybridization buffer (6x SSC, 0.1% SDS, 1 mM EDTA, 5x Denhardt's, 0.05% sodium pyrophosphate and 50 .mu.g/ml denatured and sheared salmon. .

DETDESC:

DETD(59)

The samples are hybridized with kinased oligonucleotide probes of 12-30 nucleotides under conditions which depend on the composition of the oligonucleotide. Typical conditions employ a temperature of 30.degree.-42.degree. C. for 24-36 hr with 5 ml/filter of this same oligo-hybridization buffer containing probe. The filters are washed two

DETD(59)

times for 15 min at 23.degree. C., each time with 6x SSC,. . . from 1 to 3 mismatches will be 40.degree.-70.degree. C., and can most easily be determined by successive washes of the hybridized filter. For example, the hybridized filters can be washed first at 40.degree. C., then at 50.degree. C., then at 60.degree. C., and then at 70.degree.. . .

DETDESC:

DETD(80)

For probing with cDNA probes, hybridization buffer contained 50% formamide, 5x SSC, 50 mM HEPES, pH 8.0, 5x Denhardt solution (1x Denhardt's=0.02% each polyvinyl pyrollidine, Ficoll,. . . SDS, 50 mM/ml yeast tRNA, and 50 mM sheared and denatured salmon sperm DNA. For probing with synthetic probes, the hybridization buffer contained 6x SSC, 0.1% SDS, 1 mM EDTA, 5x Denhardt's, 0.05% sodium pyrophosphate, and 15 .mu.g/ml

DETD(80)

denatured and sheared. . .

DETDESC:

DETD(82)

The . . . synthesis, as also described (oligos a and b). For reverse-transcribed RNA, prehybridization was at 42.degree. C. for 12-14 hr, and hybridization was at 42.degree. C. for 12-36 hr using 10.sup.5 cpm/filter. For probing with oligos a or b, prehybridization was at 42.degree. C. for 2-8 hours and hybridization, at 33.degree. C. for 6-12 hours using 10.sup.6 cpm/filter kinased probe. (The 33.degree. C. temperature was calculated using the formula. . . number of positive clones were obtained from both EIV-A1 and EIV-A2 libraries, but only one clone from each library which hybridized with all three probes in each of the four cases was retained for further study.

DETD(82)

DETDESC:

DETD(130)

Virus is harvested and assayed for the presence of the EIV-derived gene by DNA-DNA dot blot hybridization, as follows: cells are scraped from the dish into an Eppendorf centrifuge tube, centrifuged for 1 minute, and the cell. . .

DETDESC:

DETD(131)

Virus containing EIV gene inserts by dot blot hybridization are infected onto monolayers of 143 cells and left until a confluent cytopathic effect is

DETD(131)
obtained. The culture medium is. . .

US PAT NO: 4,508,826 L5: 9 of 10

ABSTRACT:
Disclosed is the novel bacteriophage TG1, TG1 derivatives, and the corresponding genome or nucleic acid components of such bacteriophages and derivatives of such genome, which are useful as DNA cloning vectors into organisms, such as. . . an autonomous element; (2) to serve as promoters for increasing expression of endogenous or foreign genes wherein said promoters are ligated to such genes or otherwise serve as promoters; and (3) to serve as regulatory elements for achieving control over endogenous and foreign gene expression; as cloning vectors, TG1, its deletion mutants, and other derivatives serve for the amplification and transfer of DNA sequences (genes) coding for useful functions, for example, genes necessary for the production of the antibiotic. . .

US PAT NO: 4,508,826 L5: 9 of 10

SUMMARY:

BSUM(32)

Phage . . . not confer any detectable phenotype, such as ribosomal RNA or transfer RNA, can be detected by either plaque or colony hybridization using complementary DNA as probes.

DETDESC:

DETD(19)

E. Gel Transfer Hybridization Analysis of Phage TG1 Lysogen DNA

DETD(19)

DETDESC:

DETD(20)

DNA . . . radioactive probe is prepared with DNA from purified phage by nick translation; and the probe is denatured with heat and hybridized to the DNA on the nitrocellulose at 42.degree. C. for 48 hours in the presence of formamide, ficoll, bovine serum. . .

DETDESC:

DETD(21)

The . . . lysogen (1.0 .mu.g) were digested with these enzymes and fractionated on an agarose gel. The restriction pattern was analyzed by

DETD(21)
hybridization with radioactively labelled phage DNA as descibed above. DNA from a nonlysogen contained no sequences homologous to the phage DNA. The cos fragment was missing in the lysogen and was replaced by two new fragments

hybridizing with phage DNA, which were 15 and 11 kbp in size. This result showed that the phage DNA integrated into. . .

DETDESC:

DETD(70)

DNA . . . fragment is tailed with approximately 10 to 15 deoxycytidine nucleotide residues (see Nelson and Brutlag, supra). The fragment is then hybridized with the tailed vector, and the mixture used to transfect protoplasts of S. cattleya. Phage are isolated from the resulting. . .

US PAT NO: 4,460,689 L5: 10 of 10

ABSTRACT:
Disclosed is the novel bacteriophage TG1, TG1 derivatives, and the corresponding genome or nucleic acid components of such bacteriophages and derivatives of such genome, which are useful as DNA cloning vectors into organisms, such as. . . an autonomous element; (2) to serve as promoters for increasing expression of endogenous or foreign genes wherein said promoters are ligated to such genes or otherwise serve as promoters; and (3) to serve as regulatory elements for achieving control over endogenous and foreign gene expression; as cloning vectors, TG1, its deletion mutants, and other derivatives serve for the amplification and transfer of DNA sequences (genes) coding for useful functions, for example, genes necessary for the production of the antibiotic. . .

SUMMARY:

BSUM(32)

Phage . . . not confer any detectable phenotype, such as ribosomal RNA or transfer RNA, can be detected by either plaque of colony hybridization using complementary DNA as probes.

DETDESC:

DETD(19)

E. Gel Transfer Hybridization Analysis of Phage TG1 Lysogen DNA

DETDESC:

DETD(20)

DNA . . . radioactive probe is prepared with DNA from purified phage by

DETD(20)
nick translation; and the probe is denatured with heat and hybridized to the DNA on the nitrocellulose at 42.degree. C. for 48 hours in the presence of formamide, ficoll, bovine serum. . .

DETDESC:

DETD(21)

The . . . lysogen (1.0 .mu.g) were digested with these enzymes and fractionated on an agarose gel. The restriction pattern was analyzed by hybridization with radioactively labelled phage DNA as described above. DNA from a nonlysogen contained no sequences homologous to the phage DNA. The cos fragment was missing in the lysogen and was replaced by two new fragments hybridizing with phage DNA, which were 15 and 11 kbp in size. This result showed that the phage DNA integrated into. . .

DETD(21)

DETDESC:

DETD(69)

DNa . . . fragment is tailed with approximately 10 to 15 deoxycytidine nucleotide residues (see Nelson and Brutlag, supra). The fragment is then hybridized with the tailed vector, and the mixture used to transfect protoplasts of S. cattleya. Phage are isolated from the resulting. . .

=>

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~xxxxxxxxxx`xxx`xxxxxxxxxx~x`xxxxxx`xxxxxxx`xxx`xxxPx
-2544:01-131-
Please log in:

user name: b 399,5,155
       17mar89 15:17:55 User035515 Session A598.1
             $0.15    0.005 Hrs File1
     $0.15  Estimated cost File1
     $0.06  Tymnet
     $0.21  Estimated cost this search
     $0.21  Estimated total session cost   0.005 Hrs.

System:OS  - DIALOG OneSearch

    File 399:CA SEARCH 1967-1989 UD=11010
             (Copr. 1989 by the Amer. Chem. Soc.)

    File   5:BIOSIS PREVIEWS 69-89/MAR BA8707;RRM3607
             (C.BIOSIS 1989)

    File 155:MEDLINE  66-89/APR (890402)
* Update Codes earlier than UD=8902 should not be searched in MEDLINE.
 * Use the JA= field instead (for example, S JA=8901).
        FILE 155 IS NOW BEING UPDATED TWICE A MONTH

      Set  Items  Description
      ---  -----  -----------

?ds

Set    Items    Description
S1         0    HYBRIDIZ? AND LIGAT? AND AMPLIF? AND NUCLEIC()ACID#
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    ds

Set    Items    Description
S1         0    HYBRIDIZ? AND LIGAT? AND AMPLIF? AND NUCLEIC()ACID#
S2         5    HYBRIDIZ? AND LIGAT? AND AMPLIF? AND NUCLEIC()ACID?
?
?t s2/9/all

 2/3/1      (Item 1 from file: 155)
06438242   88083242
  Cell cycle phase-specific cDNA libraries reflecting phase-specific gene
expression of Ehrlich ascites cells growing in vivo.
  Lu X; Kopun M; Werner D
  Institute of Cell and Tumor Biology, German Cancer Research Center,
Heidelberg.
  Exp Cell Res (UNITED STATES) Jan 1988, 174 (1) p199-214, ISSN
0014-4827  Journal Code: EPB

 2/3/2      (Item 2 from file: 155)
06009828   86310828
  Extrachromosomal DNA transformation of Caenorhabditis elegans.
  Stinchcomb DT; Shaw JE; Carr SH; Hirsh D
  Mol Cell Biol  Dec 1985,  5 (12) p3484-96,  ISSN 0270-7306
Journal Code: NGY
  Contract/Grant No.: GM19851; HD11761

 2/3/3      (Item 3 from file: 155)
05781709   86082709
  Introduction of cloned DNA into sea urchin egg cytoplasm: replication and
persistence during embryogenesis.
  McMahon AP; Flytzanis CN; Hough-Evans BR; Katula KS; Britten RJ; Davidson
EH
  Dev Biol  Apr 1985,  108 (2) p420-30,  ISSN 0012-1606  Journal Code:
E7T
  Contract/Grant No.: HD-05753; HD-07257

 2/3/4      (Item 4 from file: 155)
05736239   86037239
  Excision-amplification of mitochondrial DNA during senescence in
Podospora anserina. DNA sequence analysis of three unique "plasmids".
  Cummings DJ; MacNeil IA; Domenico J; Matsuura ET
  J Mol Biol  Oct 20 1985,  185 (4) p659-80,  ISSN 0022-2836
Journal Code: J6V

 2/3/5      (Item 5 from file: 155)
04544201   82087201
  Molecular cloning of DNA sequences coding for mouse embryonic globins.
  Fantoni A; Farace MG; Gambari G; Raschella G; Tripodi M
  Acta Biol Med Ger  1981,  40 (4-5) p505-10,  ISSN 0001-5318
Journal Code: OE6
?


 e au=richards, r?

Ref   Items  Index-term
E1        1  AU=RICHARDS, R. S.
E2       31  AU=RICHARDS, R. W.
E3        0 *AU=RICHARDS, R?
E4        1  AU=RICHARDS, RALPH R.
E5       21  AU=RICHARDS, RANDAL W.
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E7        10  AU=RICHARDS, RANDALL G.
E8         2  AU=RICHARDS, RANDALL GEORGE
E9         1  AU=RICHARDS, RANDY
E10        2  AU=RICHARDS, RAY S.
E11        3  AU=RICHARDS, RAYMOND
E12        9  AU=RICHARDS, RAYMOND L (ED)

          Enter P or E for more
?e au=richards, rod?

Ref    Items  Index-term
E1         3  AU=RICHARDS, ROBIN E.
E2         1  AU=RICHARDS, ROBIN L.
E3         0 *AU=RICHARDS, ROD?
E4         5  AU=RICHARDS, RODNEY M.
E5         1  AU=RICHARDS, RODNEY MARK
E6         6  AU=RICHARDS, ROGER
E7         2  AU=RICHARDS, ROGER G.
E8         1  AU=RICHARDS, ROGER K.
E9         3  AU=RICHARDS, ROLF
E10        1  AU=RICHARDS, RONALD A.
E11        1  AU=RICHARDS, RONALD F. J.
E12        2  AU=RICHARDS, RONALD L.

          Enter P or E for more
?s e4 and e5
                5  AU=RICHARDS, RODNEY M.
                1  AU=RICHARDS, RODNEY MARK
      S3        6  AU="RICHARDS, RODNEY M." OR AU="RICHARDS, RODNEY MARK"
?e au=richards, r>?

Ref    Items  Index-term
E1         1  AU=RICHARDS, R. S.
E2        31  AU=RICHARDS, R. W.
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E4         1  AU=RICHARDS, RALPH R.
E5        21  AU=RICHARDS, RANDAL W.
E6         1  AU=RICHARDS, RANDALL
E7        10  AU=RICHARDS, RANDALL G.
E8         2  AU=RICHARDS, RANDALL GEORGE
E9         1  AU=RICHARDS, RANDY
E10        2  AU=RICHARDS, RAY S.
E11        3  AU=RICHARDS, RAYMOND
E12        9  AU=RICHARDS, RAYMOND L (ED)

          Enter P or E for more
?e au=richard, r. m?

Ref    Items  Index-term
E1         2  AU=RICHARD, R. E.
E2         1  AU=RICHARD, R. M.
E3         0 *AU=RICHARD, R. M?
E4         3  AU=RICHARD, RALPH M (ED)
E5         1  AU=RICHARD, RALPH M.
E6         2  AU=RICHARD, RAYMOND
E7         1  AU=RICHARD, RAYMOND L.
E8         4  AU=RICHARD, RENE
E9         1  AU=RICHARD, REX E.
E10        1  AU=RICHARD, RICHARD R.
E11        1  AU=RICHARD, ROBERT
E12        1  AU=RICHARD, ROBERT EDWARD, JR.

          Enter P or E for more
?s e2
      S4        1  AU="RICHARD, R. M."
```

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                6  S3
                1  S4
      S5        7  S3 OR S4
?t s5/3/all

 5/3/1      (Item 1 from file: 399)
  107217615     CA: 107(23)217615s     JOURNAL
  Conformational properties of the THYME polyethers.  The bis-THYME
cylinder:  a three-dimensional analog of 18-crown-6
  AUTHOR(S): Walba, David M.; Richards, Rodney M.; Hermsmeier, Mark;
Haltiwanger, R. Curtis
  LOCATION: Dep. Chem. Biochem., Univ. Colorado, Boulder, CO, 80309-0215,
USA
  JOURNAL: J. Am. Chem. Soc.  DATE: 1987  VOLUME: 109  NUMBER: 23  PAGES:
7081-7  CODEN: JACSAT  ISSN: 0002-7863  LANGUAGE: English

 5/3/2      (Item 2 from file: 399)
  107023319     CA: 107(3)23319c     JOURNAL
  The THYME polyethers. An approach to the synthesis of a molecular knotted
ring
  AUTHOR(S): Walba, David M.; Armstrong, Joseph D., III; Perry, Ann E.;
Richards, Rodney M.; Homan, Timothy C.; Haltiwanger, R. Curtis
  LOCATION: Dep. Chem., Univ. Colorado, Boulder, CO, 80309, USA
  JOURNAL: Tetrahedron  DATE: 1986  VOLUME: 42  NUMBER: 6  PAGES: 1883-94
  CODEN: TETRAB  ISSN: 0040-4020  LANGUAGE: English

 5/3/3      (Item 3 from file: 399)
  106117881     CA: 106(15)117881m     JOURNAL
  Structure and activity of recombinant human interferon-.gamma. analogs
  AUTHOR(S): Hsu, Yeuh Rong; Ferguson, Betsy; Narachi, Michael; Richards,
Rodney M.; Stabinsky, Yitzhak; Alton, N. Kirby; Stebbing, Nowell; Arakawa,
Tsutomu
  LOCATION: Amgen, Thousand Oaks, CA, 91320, USA
  JOURNAL: J. Interferon Res.  DATE: 1986  VOLUME: 6  NUMBER: 6  PAGES:
663-70  CODEN: JIREDJ  ISSN: 0197-8357  LANGUAGE: English

 5/3/4      (Item 4 from file: 399)
  102113463     CA: 102(13)113463w     DISSERTATION
  A "strip-strategy" for the synthesis of molecular cylinders and Moebius
strips:  crown ether rings fused by the tetrahydroxymethylethylene (THYME)
unit
  AUTHOR(S): Richards, Rodney Mark
  LOCATION: Univ. Colorado, Boulder, CO, USA
  DATE: 1984  PAGES: 96 pp.  CODEN: DABBBA  LANGUAGE: English  CITATION:
Diss. Abstr. Int. B 1985, 45(7), 2164  AVAIL: Univ. Microfilms Int., Order
No. DA8422641

 5/3/5      (Item 5 from file: 399)
  97023763     CA: 97(3)23763t     JOURNAL
  Total synthesis of the first molecular Moebius strip
  AUTHOR(S): Walba, David M.; Richards, Rodney M.; Haltiwanger, R. Curtis
  LOCATION: Dep. Chem., Univ. Colorado, Boulder, CO, 80309, USA
  JOURNAL: J. Am. Chem. Soc.  DATE: 1982  VOLUME: 104  NUMBER: 11  PAGES:
3219-21  CODEN: JACSAT  ISSN: 0002-7863  LANGUAGE: English

 5/3/6      (Item 6 from file: 399)
  95150629     CA: 95(17)150629n     JOURNAL
  Strategy for the synthesis of cylindrical macropolycyclic hosts with
hydrophilic interior surfaces:  crown ether rings fused by the
tetrahydroxymethylethylene (THYME) unit
  AUTHOR(S): Walba, David M.; Richards, Rodney M.; Sherwood, Steven P.;
Haltiwanger, R. Curtis
  LOCATION: Dep. Chem., Univ. Colorado, Boulder, CO, 80309, USA
  JOURNAL: J. Am. Chem. Soc.  DATE: 1981  VOLUME: 103  NUMBER: 20  PAGES:
6213-15  CODEN: JACSAT  ISSN: 0002-7863  LANGUAGE: English
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5/3/7 (Item 7 from file: 399)
67042345 CA: 67(9)42345e JOURNAL
Inhibition of the anticoagulant activity of neodymium chloride by sodium pyrophosphate
AUTHOR(S): Gabbiani, Giulio; Solymoss, Bela; Richard, R. M.
LOCATION: Inst. Med., Univ. Montreal, Montreal, Can.
JOURNAL: Arzneim.-Forsch. DATE: 1967 VOLUME: 17 NUMBER: 4 PAGES: 505-7 CODEN: ARZNAD LANGUAGE: English
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17mar89 15:27:49 User035515 Session A598.2
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$19.39 Estimated cost this search
$19.60 Estimated total session cost 0.170 Hrs.
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TYMNET: call cleared by request

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